

That Which is Claimed is:

1. A method of identifying an inhibitor of retrovirus protease activity, comprising:

5 (a) providing a nucleic acid that encodes a retrovirus GagPol or a fragment thereof comprising a protease, a protease cleavage site, a tether and a detectable moiety, wherein either the tether or the detectable moiety is located N-terminal to the cleavage site and the other is located C-terminal to the protease cleavage site;

10 (b) expressing the nucleic acid to produce the retrovirus GagPol or fragment thereof;

(c) binding the retrovirus GagPol or fragment thereof to a substrate comprising a binding partner for the tether such that the retrovirus GagPol or fragment thereof is bound via the tether to the substrate;

15 (d) contacting the retrovirus GagPol or fragment thereof with a candidate compound;

(e) removing released proteolytic products comprising the detectable moiety; and

20 (f) detecting the level of the detectable moiety bound to the substrate wherein persistence of the detectable moiety is indicative of an inhibitor of retrovirus protease activity.

2. The method according to Claim 1, wherein the retrovirus is a Human Immunodeficiency Virus (HIV).

25 3. The method according to any one of Claims 1 to 2, wherein the retrovirus is a resistant retrovirus strain.

30 4. The method according to any of Claims 1 to 3, wherein the nucleic acid encodes a retrovirus GagPol fragment comprising the retrovirus protease and transframe protein.

5. The method according to Claim 4, wherein the fragment further comprises the retrovirus nucleocapsid protein.

6. The method according to Claim 5, wherein the fragment further
5 comprises the retrovirus p2 protein.

7. The method according to Claim 6, wherein the fragment further comprises the retrovirus capsid protein.

10 8. The method according to Claim 7, wherein the fragment further comprises the retrovirus matrix protein.

9. The method according to any one of Claims 1 to 8, wherein the
nucleic acid encodes a retrovirus GagPol fragment comprising the retrovirus
15 protease and the retrovirus reverse transcriptase.

10. The method according to Claim 9, wherein the fragment further comprises the retrovirus integrase.

20 11. The method according to Claim 1, wherein the nucleic acid encodes the retrovirus GagPol.

12. The method according to any one of Claims 1 to 11, wherein the
tether is an epitope within the retrovirus GagPol or fragment thereof.
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13. The method according to Claims 7 or 12, wherein the tether is an epitope within the retrovirus capsid protein.

14. The method according to any one of Claims 1 to 13, wherein the
30 binding partner for the tether is an antibody.

15. The method according to any one of Claims 1 to 14, wherein the detectable moiety is selected from the group consisting of luciferase,

hemagglutinin antigen, maltose binding protein, c-myc, FLAG epitope, glutathione-S-transferase, fluorescent moiety, β -glucuronidase, alkaline phosphatase and β -galactosidase.

5 16. The method according to any one of Claims 1 to 14, wherein the detectable moiety is an epitope within the retrovirus GagPol or fragment thereof.

10 17. The method according to any one of Claims 1 to 16, wherein the method comprises an ELISA-based assay.

15 18. The method according to any one of Claims 1 to 17, wherein said detecting step further comprises comparing the level of the detectable moiety bound to the substrate with a predetermined standard.

19. The method according to Claim 18, wherein the predetermined standard is the level of detectable moiety detected in the presence of a known retrovirus protease inhibitor.

20 20. A kit for identifying inhibitors of retrovirus protease activity, comprising:

25 (a) a nucleic acid that encodes a retrovirus GagPol or a fragment thereof comprising a protease, a protease cleavage site, a tether and a detectable moiety, wherein either the tether or the detectable moiety is located N-terminal to the cleavage site and the other is located C-terminal to the protease cleavage site, such that cleavage at the protease cleavage site results in release of a proteolytic product comprising the detectable moiety; and

30 (b) a substrate comprising a binding partner for the tether.

21. The kit according to Claim 20, wherein the kit further comprises a rabbit reticulocyte lysate.

22. The kit according to any one of Claims 20 to 21, wherein the kit further comprises a reagent for detecting the detectable moiety.

23. The kit according to any one of Claims 20 to 22, wherein the
5 retrovirus is a Human Immunodeficiency Virus (HIV).

24. The kit according to any of Claims 20-23, wherein the retrovirus is a resistant retrovirus strain.

10 25. The kit according to any of Claims 20-24, wherein the nucleic acid encodes a retrovirus GagPol fragment comprising the retrovirus protease and transframe protein.

26. The kit according to Claim 25, wherein the fragment further
15 comprises the retrovirus nucleocapsid protein.

27. The kit according to Claim 26, wherein the fragment further comprises the retrovirus p2 protein.

20 28. The kit according to Claim 27, wherein the fragment further comprises the retrovirus capsid protein.

29. The kit according to Claim 28, wherein the fragment further
25 comprises the retrovirus matrix protein.

30. The kit according to any one of Claims 20 to 29, wherein the nucleic acid encodes a retrovirus GagPol fragment comprising the retrovirus protease and the retrovirus reverse transcriptase.

30 31. The kit according to Claim 30, wherein the fragment further comprises the retrovirus integrase.

32. The kit according to Claim 20, wherein the nucleic acid encodes a retrovirus GagPol.

33. The kit according to any one of Claims 20 to 32, wherein the tether
5 is an epitope within the retrovirus GagPol or fragment thereof.

34. The kit according to Claims 28 or 33, wherein the tether is an epitope within the retrovirus capsid protein.

10 35. The kit according to any one of Claims 20 to 32, wherein the binding partner is an antibody.

36. A nucleic acid that encodes a retrovirus GagPol or a fragment thereof comprising a protease, a protease cleavage site, an exogenous tether
15 and an exogenous detectable moiety, wherein either the tether or the detectable moiety is located N-terminal to the protease cleavage site and the other is located C-terminal to the protease cleavage site.

20 37. A vector comprising the nucleic acid of claim 36.